Mathematical Modeling of Growth of Non-O157 Shiga Toxin-Producing *Escherichia coli* in Raw Ground Beef

Lihan Huang, Shu-I Tu, John Phillips, and Pina Fratamico

Abstract: The objective of this study was to investigate the growth of Shiga toxin-producing *Escherichia coli* (STEC, including serogroups O45, O103, O111, O121, and O145) in raw ground beef and to develop mathematical models to describe the bacterial growth under different temperature conditions. Three primary growth models were evaluated, including the Baranyi model, the Huang 2008 model, and a new growth model that is based on the communication of messenger signals during bacterial growth. A 5 strain cocktail of freshly prepared STEC was inoculated to raw ground beef samples and incubated at temperatures ranging from 10 to 35 °C at 5 °C increments. Minimum relative growth (<1 $\log_{10}$ cfu/g) was observed at 10 °C, whereas at other temperatures, all 3 phases of growth were observed. Analytical results showed that all 3 models were equally suitable for describing the bacterial growth under constant temperatures. The maximum cell density of STEC in raw ground beef increased exponentially with temperature, but reached a maximum of 8.53 $\log_{10}$ cfu/g of ground beef. The specific growth rates estimated by the 3 primary models were practically identical and can be evaluated by either the Ratkowsky square-root model or a Bělehrádek-type model. The temperature dependence of lag phase development for all 3 primary models was also developed. The results of this study can be used to estimate the growth of STEC in raw ground beef at temperatures between 10 and 35 °C.

Keywords: ground beef, kinetic analysis, Shiga toxin-producing *Escherichia coli*, predictive microbiology

Practical Application: Incidents of foodborne infections caused by non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have increased in recent years. This study reports the growth kinetics and mathematical modeling of STEC in ground beef. The mathematical models can be used in risk assessment of STEC in ground beef.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are a group of foodborne pathogens that have caused outbreaks and sporadic cases of human infections worldwide (Carter and others 1987; Caprioli and others 1994; Ludwig and others 2002). Also known as verotoxin-producing *E. coli* (VTEC), STEC can cause human illnesses ranging from mild to bloody diarrhea, hemorrhagic colitis (HC), and life-threatening hemolytic uremic syndrome (Bonardi and others 2005; Gyles 2007). Although *E. coli* O157:H7 has been the most prominent STEC serotype (Fratamico and others 2004), numerous non-O157 STEC serogroups have been identified, and an increasing number of outbreaks and human illnesses caused by non-O157 STEC have been reported worldwide (Caprioli and others 1994; Bonardi and others 2005; Brooks and others 2005; Ethelberg and others 2009).

Ruminants are the major reservoir of STEC, with cattle as the most significant carrier of STEC in North America (Gyles 2007). Among all STEC serotypes, *E. coli* O157:H7 is usually associated with the most severe forms of foodborne infections (Brooks and others 2005; Tozzi and others 2003; Rivero and others 2010; Bosilevac and Koohmaraie, 2011). Other common disease-causing non-O157 STEC serogroups and relative percentage of reported infections are O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%), and O145 (5%) (Brooks and others 2005). According to Bosilevac and Koohmaraie (2011), numerous O-serogroups were found in commercial ground beef, with O113, O8, O22, O117, O163, O174, O171, O116, and O20 being the most frequently identified serogroups.

Although most research concerning non-O157 STEC focuses on the detection of these pathogens in foods, the objective of this work was to investigate the growth kinetics of some non-O157 STEC in ground beef. The main goal of this research was to examine how rapidly these bacteria grow under various temperatures and to develop mathematical models to describe the bacterial growth behaviors. The information collected from this investigation may be useful for the food industry and regulatory agencies to conduct risk assessment of ground beef exposed to various temperature abuse conditions.

Materials and Methods

Bacterial strains

Five Kanamycin-resistant strains (serotypes O45, O111:H7, O103:H2, O121, and O145:HNM) of non-O157 STEC strains were kindly provided by Dr. John B. Luchansky of the Eastern

MS 20111178 Submitted 9/29/2011, Accepted 1/2/2012. *Authors are with U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, U.S.A. Direct inquiries to author Huang (E-mail: lihan.huang@ars.usda.gov).*

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Regional Research Center of the USDA Agricultural Research Service. The preparation and application of these strains of STEC were reported in Luchansky and others (2011). The bacterial strains were propagated by inoculating and aerobically incubating the individual stock cultures in 10 mL of Brain Heart Infusion (BHI, BD, Sparks, Md., U.S.A.) broth at 37 °C overnight. The propagated cultures were streaked onto Tryptic Soy agar (TSA, BD) plates supplemented with 100 μg/mL kanamycin (K-1876, Sigma-Aldrich, St. Louis, Mo., U.S.A., Lot #110K1192), and stored in a refrigerator set at 10 °C. This temperature was slightly above the minimum growth temperature for most *E. coli* and helped prevent cell death and injury during refrigerated storage. The bacterial cultures were regularly (every 2 to 3 weeks) transferred to maintain cell viability.

One day before the experiment, working cultures were prepared by incubating 10 mL of BHI broth individually inoculated with each STEC strain overnight (18 to 20 h) at 37 °C with mild agitation. The working bacterial cultures were harvested by centrifugation (2400 × *g* for 10 min at 4 °C), washed once with 5 mL 0.1% peptone water (PW, BD), and resuspended in 1 mL PW. The cultures were combined to form a 5 mL cocktail.

Sample preparation and inoculation

Ground beef (90% lean) was purchased from a local grocery store. The ground beef samples were divided into 5 ± 0.1 g portions and packaged into filter bags (Whirl-Pak®, 7 oz, 95 mm × 180 mm × 0.08 mm, NASCO—Fort Atkinson, Fort Atkinson, Wis., U.S.A.). With the openings closed, the filter bags containing beef samples were frozen at −20 °C and used within 2 weeks.

One night before the experiment, the frozen samples were retrieved from the freezer and thawed overnight in a refrigerator (4 to 5 °C). The thawed samples were inoculated with 0.1 mL of the bacterial cocktail, which was diluted before inoculation. The final concentration of STEC in the beef was approximately 10^3 cfu/g. The inoculated samples were pulsated for 1 min in a mechanical stomacher (Model BagMixer® 100W, Interscience Co., France) at maximum speed.

Growth study

After inoculation, the samples were properly labeled and incubated at 10, 15, 20, 25, 30, or 35 °C. The incubation time ranged from 24 to 336 h, depending on the incubation temperature. The incubating samples were periodically retrieved to enumerate STEC. The sampling frequencies were determined by the incubation temperature, and ranged from every hour to every 24 h. At each predetermined sampling time, samples were retrieved for enumeration of bacteria. The growth experiments were replicated at least 2 times for each temperature condition. No STEC was detected from the raw ground beef samples.

Enumeration of bacteria

To each sample, 10 mL of PW was added. The samples were pulsated for 6 min at maximum speed in the previously mentioned mechanical stomacher. A small volume (0.1 or 1 mL) of the liquid portion was plated, either directly or after serial dilution, onto TSA plates supplemented with kanamycin (100 μg/mL), to recover and enumerate STEC cells. The cell counts were converted to natural logarithm of colony-forming units per gram, or ln cfu/g, of ground beef.

Mathematical modeling

Baranyi and Huang models. Three primary growth models were used to describe bacterial growth curves. The 1st model was the Baranyi model (Baranyi and others 1993a, 1993b; Baranyi and Roberts, 1994). For this model, with an initial concentration of Y_0, the natural logarithm of bacterial count (Y) is expressed as a function of time (t), and takes the form of

$$Y = Y_0 + \mu t + \ln(e^{-\mu t} + e^{-h_0} - e^{-\mu t - h_0}) - \ln\left(1 + \frac{e^{\mu t - h_0} - e^{h_0}}{e^{Y_{\max} - Y_0}}\right)$$

$$\lambda = \frac{h_0}{\mu} \quad (1)$$

The 2nd growth model was the Huang 2008 model (Huang, 2008, 2010). This model was developed to account for the 3 growth phases of bacteria under constant temperature conditions.

$$\begin{aligned} Y &= Y_0 + Y_{\max} - \ln\{\exp(Y_0) + [\exp(Y_{\max}) - \exp(Y_0)] \\ &\quad \times \exp[-\mu \times B(t)]\} \\ B(t) &= t + \frac{1}{25}\ln\frac{1 + \exp[-25(t - \lambda)]}{1 + \exp(25\lambda)} \end{aligned} \quad (2)$$

In Eq. 1 and 2, μ is the specific growth rate in ln cfu/g, λ is the lag phase of a growth curve under a constant temperature, and h_0 is used to represent the physiological state of the bacteria. In Eq. 2, $B(t)$ is the time adjustment function used to account for the lag phase duration.

Both Baranyi and Huang 2008 models were developed by characterizing the formation of lag phase before exponential growth. The Baranyi model is based on the hypothesis that bacteria need time to accumulate critical substances before exponential growth starts. The Huang 2008 model is based on the hypothesis that there is no growth in the lag phase (Huang 2008). Both models use the logistic growth model for the exponential and stationary phases.

Development of a new growth model. The new growth model is based on the observation that a lag phase can be induced if actively dividing bacterial cells, which may contain all necessary materials internally, are exposed to a new environment that may contain all necessary nutrients for bacterial growth. For developing this new model, it is assumed that a bacterial cell may contain all necessary materials internally. The bacterial cells always attempt to communicate with the environment and with each other by sending and receiving some messenger signal compounds. One potential mechanism of inter-bacterial communication may be quorum sensing and secretion of communication signals such as auto-inducers (Barrios and others 2006; Gobbetti and others 2007; Zhu and Pet 2008). The communication with the environment and among the bacterial cells controls the growth process. It is also assumed that the bacteria begin to communicate immediately after being exposed to a new environment. In this study, it is hypothesized that bacterial communication occurs among STEC cells, and the bacterial communication process can be denoted by a hypothetical communication function (f). This hypothetical communication function (f) is zero immediately after the bacteria are exposed to a new environment, and is equal to 1 when the communication is fully established, which usually occurs at the peak of bacterial growth. This communication process can be described by a simple mathematical equation (Eq. 3).

$$f = 1 - e^{-kt} \quad (3)$$

In Eq. 3, the parameter k defines the rate at which the bacteria communicate with the environment. For normal bacterial growth, it is assumed that the bacteria increase in population by binary fission, and this process is governed by

$$\frac{dC}{dt} = f\mu C \quad (4)$$

In Eq. 4, C is the bacterial concentration in cfu, or colony forming units; and μ is the specific growth rate. Equation 4 effectively governs the multiplication of the bacterial population, ranging from lag to exponential phases. As the bacterial density increases, the increase in the bacterial population is gradually inhibited, which can be described by an inhibition term $(1 - \frac{C}{C_{max}})$, where C_{max} is the maximum cell concentration. Therefore, the

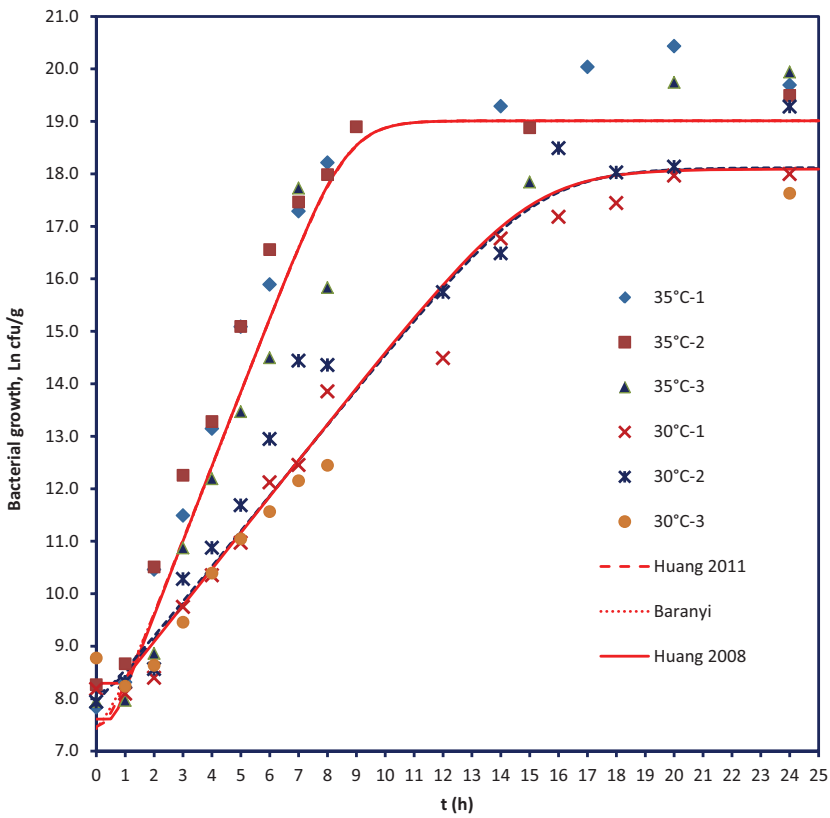

Figure 1–Growth of STEC in ground beef at 30 and 35 °C and curve-fitting with 3 growth models.

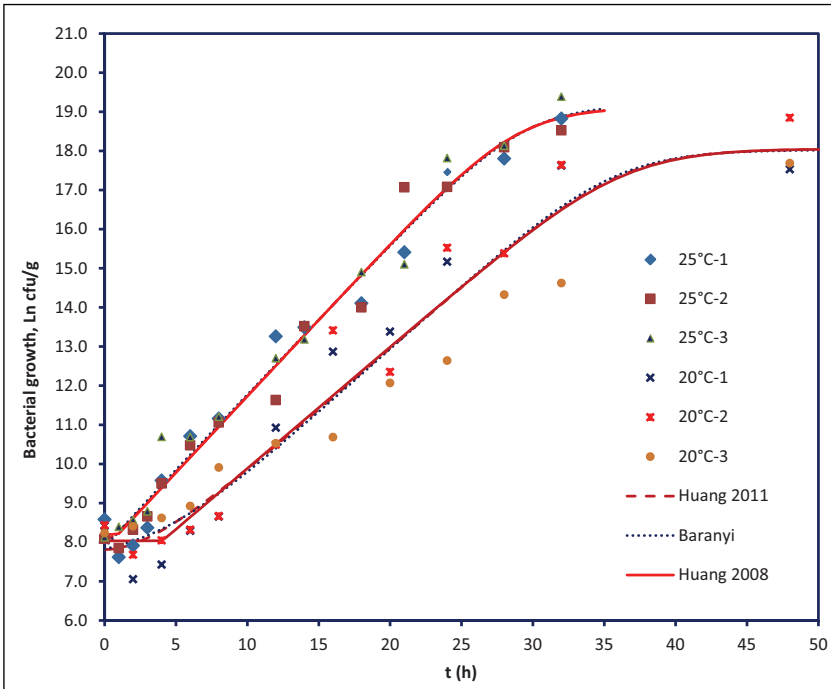

Figure 2–Growth of STEC in ground beef at 20 and 25 °C and curve-fitting with 3 growth models.

entire growth process can be described by

$$\frac{dc}{dt} = (1 - e^{-kt})\,\mu C\left(1 - \frac{C}{C_{max}}\right) \quad (5)$$

The above differential equation can be solved by separation of variables to yield an analytical solution, which is expressed in Eq. 6. For simplicity, this model is called the Huang 2011 model from this point forward.

$$\begin{aligned} Y &= Y_0 + \alpha(t) - ln\left[1 - \frac{1 - e^{\alpha(t)}}{e^{Y_M - Y_0}}\right] \\ \alpha(t) &= \mu t + \frac{\mu}{k}[e^{-kt} - 1] \end{aligned} \quad (6)$$

Effect of temperature on bacterial growth. The effect of temperature on the growth rate (μ) of STEC in ground beef was evaluated using the Ratkowsky square-root model (Eq. 7) and a Bělehrádek-type model (Eq. 8) modified from Huang (2010).

$$\sqrt{\mu} = \alpha(T - T_0) \quad (7)$$

$$\sqrt{\mu} = \alpha(T - T_{min})^{0.75} \quad (8)$$

Curve-fitting

The growth data collected from the growth experiments were used to develop primary growth models. As the initial concentrations were similar, the growth data obtained at the same growth temperature conditions were combined to form a single data set for curve-fitting. The curve-fitting was performed using the nonlinear regression procedure (NLIN) in the SAS software package (SAS Version 9.2, Cary, S.C., U.S.A.).

To compare the accuracy between the models, the mean squared error (MSE) of each growth curve was calculated using the following equation:

$$MSE = \frac{\sum (Y_{raw\ data} - Y_{estimated})^2}{df} \quad (9)$$

In Eq. 9, df ($= n - p$) represents the degree of freedom, which is the number of observations (n) in each growth curve minuses the number of estimated parameters (p). The procedure ANOVA, or Analysis of Variance, of SAS (Version 9.2, SAS) was used to compare the effect of different models on MSE. The null hypothesis, H_0, was that the means of MSE were equal among all 3 models.

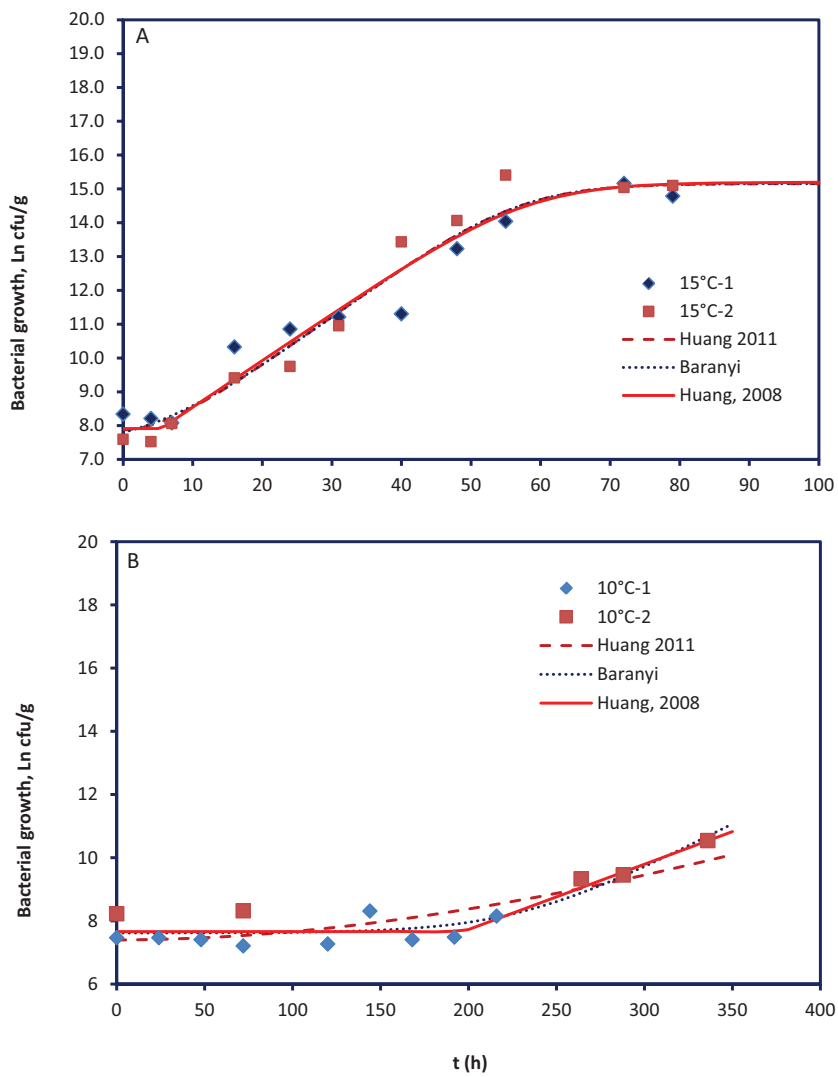

Figure 3–Growth of STEC in ground beef at 10 and 15 °C and curve-fitting with 3 growth models.

Results and Discussion

Bacterial growth curves and curve-fitting

The average count of the bacterial background flora was 6.8 $\log_{10}$ cfu/g, with a standard deviation of 0.8 $\log_{10}$ cfu/g (n = 16). The average initial concentration of STEC in ground beef was 3.5 $\log_{10}$ cfu/g. As the ground beef samples inoculated with STEC cells were exposed to the different growth temperature conditions (15–35 °C), the STEC bacterial cells began to grow after different lag phases and at different growth rates (Figure 1 to 3). Although the background flora concentration was on average 3.4 times higher in magnitude than the inoculated STEC concentration, the STEC cells still grew well in ground beef. At 10 °C, however, the growth of STEC was outcompeted by background microflora, and did not develop into full growth curves (Figure 3B). The relative growth of STEC in raw ground beef was less than 1 $\log_{10}$ cfu/g, and the growth was practically insignificant. This observation was similar to another study reported by Huang

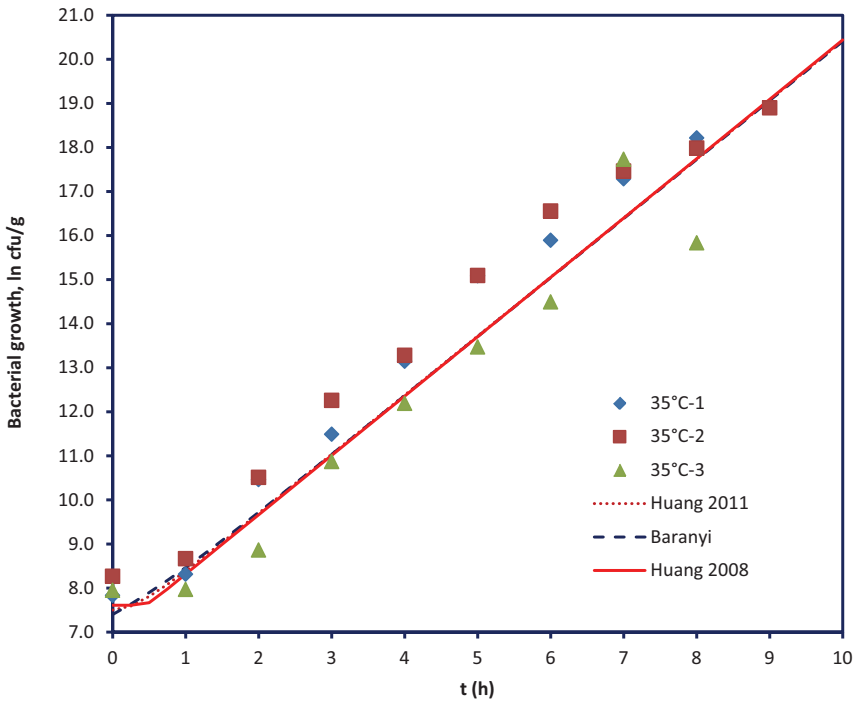

Figure 4–Curve-fitting for the incomplete growth curves (lag and exponential phases) of STEC at 35 °C.

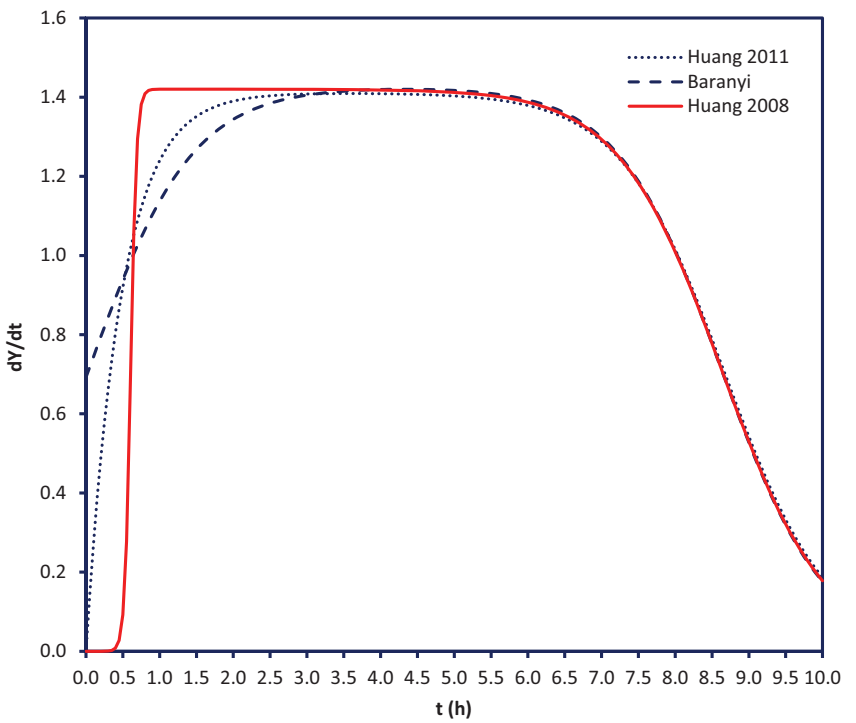

Figure 5–The real time growth rate calculated from 3 models for the growth curve at 35 °C shown in Figure 1.

(2010), in which *E. coli* O157:H7 was inoculated to mechanically tenderized beef. *E. coli* is a mesophilic microorganism, and it may survive, but does not grow well at refrigerated temperature conditions. In raw ground beef samples, numerous background microflora may exist and those that are psychrotrophic may grow better at refrigerated temperatures (Gounot 1986). If sterile ground beef had been used at 10 °C, it is possible that STEC would have grown unrestrictedly, and full growth curves could be developed.

For experiments conducted at temperatures of 15, 20, 25, 30, and 35 °C, STEC grew well and full growth curves were developed (Figure 1 to 3A). The growth rate was higher with higher growth temperatures, and the trend was reversed for lag phase (that is, lags decreased with higher temperatures). In these figures, each growth curve represents an independent study. Because the initial bacterial inoculum was relatively consistent at each temperature condition, the growth behavior at each temperature was similar. Therefore, it is possible to combine the growth curves obtained at the same temperature to derive kinetic parameters for STEC in ground beef. Therefore, all growth curves obtained at the same temperatures were combined for curve-fitting to determine the averaged growth parameters (μ, λ, α, and k, respectively) for each temperature.

All 3 models can be used to fit the growth curves obtained at each temperature (Figure 1 to 3A). At 10 °C, only the first 2 phases (lag phase and early stage of exponential phase) of the growth curves were observed. As illustrated in Figure 1 to 3, all 3 models were suitable for curve-fitting. The Huang 2008 model was clearly distinguished from the Barany model and the Huang 2011 model by possessing and demonstrating a lag phase in all growth curves. By visual inspection, the performance of the Baranyi and the Huang 2011 models are basically identical for all 3 phases from lag phase to stationary phase for full growth curves. The performance of all 3 models is almost identical for the exponential and stationary phases for all growth curves.

The growth curves obtained at 10 °C were incomplete ones that did not contain all 3 phases. Therefore, the full models cannot be used to fit the growth curves. For growth curves without stationary phase, models for a partial growth curve must be used. For the Huang 2008 model, the partial model is written as

$$Y = Y_0 + \mu\left\{t + \frac{1}{25}\ln\frac{1+\exp\left[-25\left(t-\lambda\right)\right]}{1+\exp\left(25\lambda\right)}\right\} \quad (10)$$

It is necessary to point out that the maximum value allowable for lag phase (λ) for Eq. 2 and 10 is approximately 28 h if the unit of time (t) is hour (h) in these equations. Exp(25×28) is a huge number, and usually beyond the upper limit of many of computing systems. To utilize this model for lag phase longer than 28 h, it is necessary to convert the time unit from hours to days by diving t by 24. After the time conversion, the model converged easily, as demonstrated in Figure 3B.

The Baranyi model also can be used to describe partial growth curves (Eq. 11). For the Baranyi model, there is no limitation on the unit of time, as none of the exponential values in the equation would exceed the physical limit of the computing system. This Baranyi model also converged easily.

$$Y = Y_0 + \mu t + \ln[\exp\left(-\mu t\right) + \exp\left(-h_0\right) - \exp\left(-\mu t - h_0\right)] \quad (11)$$

The new growth model also can be modified to fit the partial growth curves, resulting in a simplified model (Eq. 12). This equation did not converge when the growth data obtained at 10 °C were directly used for nonlinear regression due to the limited number of data points. To obtain the estimate of μ and k for the Huang 2011 model, the average of the μ values obtained from the Baranyi and Huang 2008 models were used to help the model converge for Eq. 12. The nonlinear regression converged and the dotted curve shown in Figure 3B represents the results for the Huang 2011 model.

$$Y = Y_0 + \mu t + \frac{\mu}{k}[\exp\left(-kt\right) - 1] \quad (12)$$

The curve-fitting results from nonlinear regression of the growth data obtained at 10 °C clearly reveal the nature of the 3 models evaluated in this study. The Baranyi and Huang 2008 models were developed by characterizing the formation of lag phase for a growth curve. Therefore, even with the limited

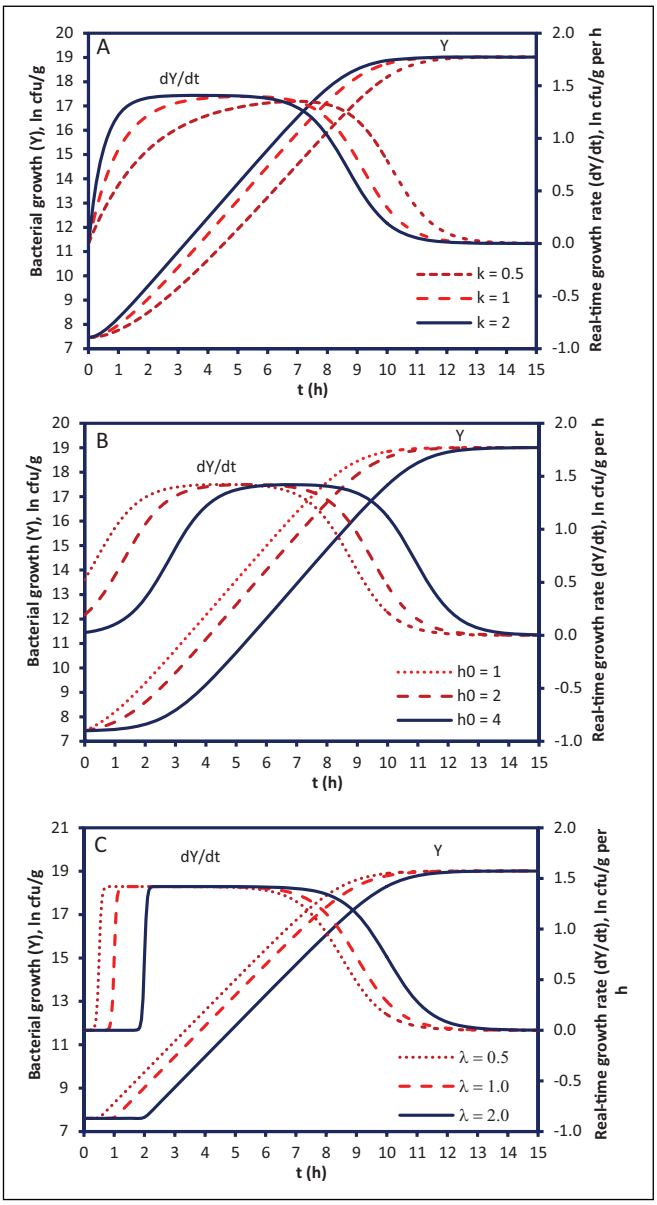

Figure 6–Effect of lag phase parameters (A: k for the Huang 2011 model; B: h_0 for the Baranyi model; and C: λ for the Huang 2008 model) on real-time bacterial growth rate (μ_{max} = 1.41, T = 35 °C).

number of data points, the Baranyi and Huang 2008 models can easily converge to obtain model coefficients with limited data points in the lag and exponential phases. The Huang 2011 model, however, is based on the hypothetical communication of messenger signals between the cells and surrounding environment. If sufficient data points are available, all 3 models are capable of describing partial growth curves. Figure 4 demonstrates the application of all 3 models for curve-fitting of partial growth curves using the 35 °C growth data shown in Figure 1, but without including the stationary phase.

Characteristics of primary growth models

Figure 5 illustrates the real-time bacterial growth rate calculated from all 3 models based on the growth models obtained at 35 °C (Figure 1). This figure clearly demonstrates the difference and similarity in defining the growth curves, particularly at the initial stage of bacterial growth where the lag phase occurs. For the Baranyi model, true to its original hypothesis, the initial growth rate (μ at $t = 0$) was not zero as the bacterial growth would resume from the end-point of its prior history in the new environment. For the Huang (2008) model, it assumes, by the definition of lag

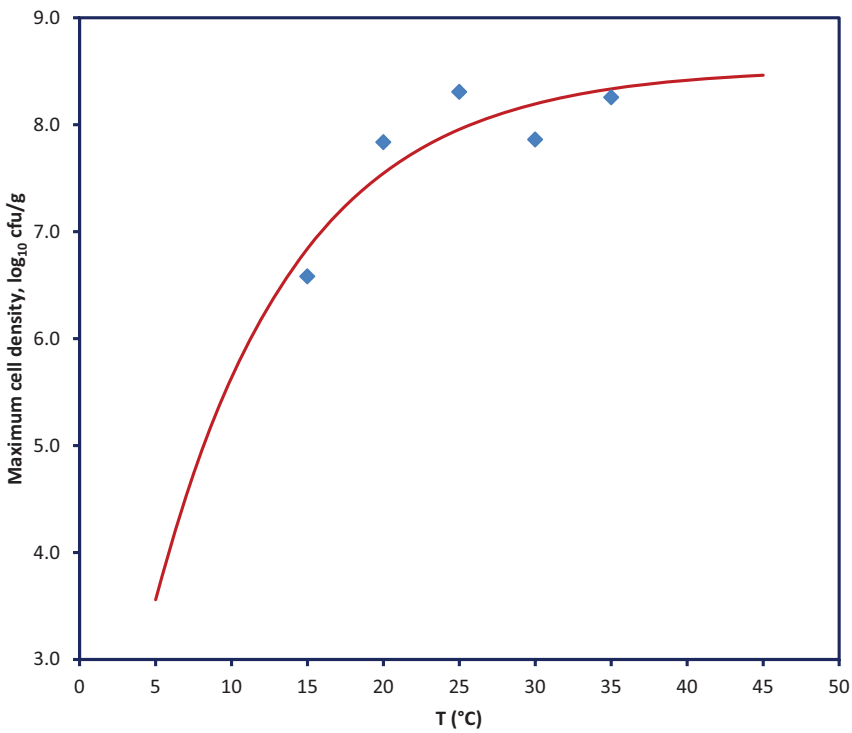

Figure 7–Effect of temperature on maximum cell density of STEC in raw ground beef.

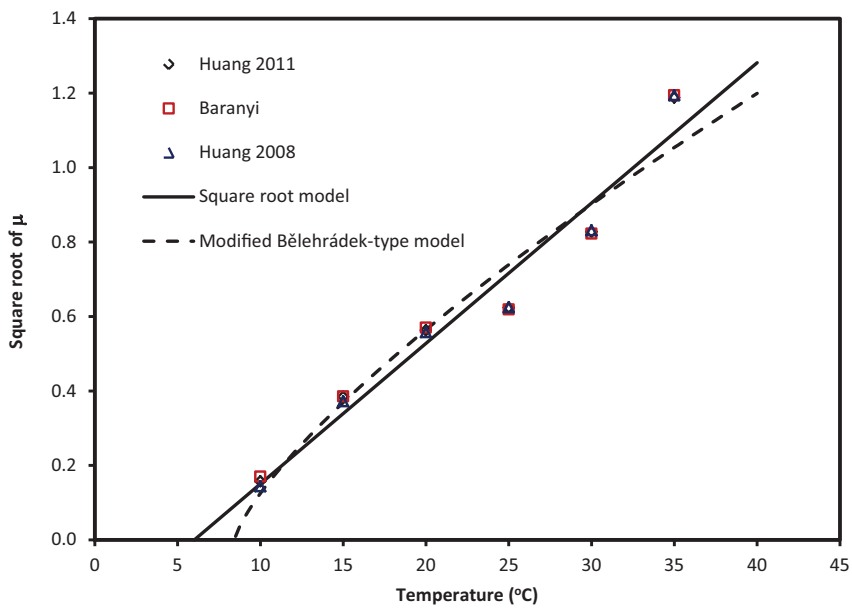

Figure 8–Effect of temperature on specific growth rate of STEC in ground beef and secondary models.

phase, that no growth would occur in this initial stage until the bacteria adjust to the new environment. Therefore, the growth rate is zero at $t = 0$ and stays so until the lag phase is over. For the Huang 2011 model, it assumes that the bacteria start to communicate with the surrounding environment immediately after exposure, and the initial growth rate is also zero. For all 3 models, the growth rates were almost identical to each other as the bacterial growth approached the stationary phase.

Figure 6 simulates the effect of lag phase parameters (k for the Huang 2011 model, h_0 for the Baranyi model, and λ for the Huang 2008 model) on real-time bacterial growth rates. For all these growth curves, the specific growth rate (μ_{max}) was set as 1.41 log cfu/g per h, which was the μ_{max} at of STEC in ground beef at 35 °C. In Figure 6A, 3 values of k are used to represent different levels of the hypothetical cellular communication and their effect on bacterial growth. The decrease in k apparently reduces the real-time growth rate of bacteria by inducing prolonged lag phases, but it does not affect the maximum cell density. Figure 6A illustrates that the hampered bacterial communication may delay the bacterial growth. According to Figure 6A, k affects the real-time growth rates.

Figure 6B simulates the effect of h_0, or the physiological state, on the bacterial growth and the corresponding growth rates for STEC in ground beef. As illustrated in Figure 6B, the values of h_0 affect the initial growth rates, and the duration of the lag phases increases with h_0. The Baranyi growth rate curves (dY/dt) appear to be symmetric with respect to the inflection points (IP) of the curves, whereas the growth curves (Y) appear mirror-symmetric with respect to IPs. With increasing h_0 values, the growth curves and the real-time growth rate curves are just parallelly moved to the right along the t axis. For the Huang 2008 model, similar properties can be observed (Figure 6C). With increasing λ values, both growth curves and growth rates are moved in parallel to the right along the horizontal axis. The Huang 2008 growth model is not symmetric.

MSE, or mean squared error, is an estimator for the difference between the measured data and their predicted values. A zero MSE would mean that a model is in perfect agreement with the measured data. In a selection of a model from multiple models, a model with a smaller MSE is preferable. The calculated average MSE was 0.4831, 0.4937, and 0.5027, for the Huang 2008 model, the Huang 2011 model, and Baranyi model, respectively, for experimental data collected at temperatures above 10 °C. According to the ANOVA results of the MSE, there was no significant difference among the 3 models ($P = 0.99$), suggesting that all 3 models were equally suitable for describing the experimental growth curves.

The maximum cell densities estimated by the growth models were identical for growth curves at each temperature above 10 °C, and each had very small standard errors (0.1 $\log_{10}$ cfu/g). It appears that the growth temperature affects the attainment of maximum cell density for STEC in raw ground beef (Figure 7), and the temperature effect can be described using a simple exponential equation, which is expressed in Eq. 13 ($R^2 = 0.993$), where C_{max} is the maximum cell concentration (cfu/g) and T is the temperature in °C.

$$\log_{10}(C_{max}) = 8.53[1 - \exp(-0.108T)] \quad (13)$$

Effect of temperature on growth rate and lag phase

As the specific growth rates (μ) estimated by all 3 primary models were practically identical, the data were combined to derive the secondary models, and the results are illustrated in Figure 8. The coefficient a is 0.0377 ± 0.002 (mean ± approximate standard error [ASE]) and 0.0901 ± 0.004 for the Ratkowsky square root model (Eq. 7) and the modified Bělehrádek-type model (Eq. 8), respectively, and corresponding T_0 and T_{min} are 6 ± 1 °C and 8.5 ± 0.8 °C. The MSE is 0.00522 for the Ratkowsky model, and 0.00739 for the modified Bělehrádek-type model.

In the Baranyi model (Eq. 1), the lag phase of a growth curve is defined by the physiological state (h_0) and the growth rate (μ). For the Huang 2008 model, the lag phase is explicitly defined (Eq. 2). For the Huang 2011 model, however, the lag phase is not explicitly expressed in the equation, but is implicitly defined by k in the model. Therefore, it is not possible to directly calculate the lag phase using this new model. The determination of the lag phase using this model would have to rely on the traditional microbiological definition method (Buchanan and Solberg 1972). Nevertheless, the effect of temperature on k in the new growth model can be described by a simple logistic model (Eq. 14), as illustrated in Figure 9A.

$$k = 0.0658 + \frac{1.941}{1 + \exp[-0.8837(T - 22.42)]} \quad (14)$$

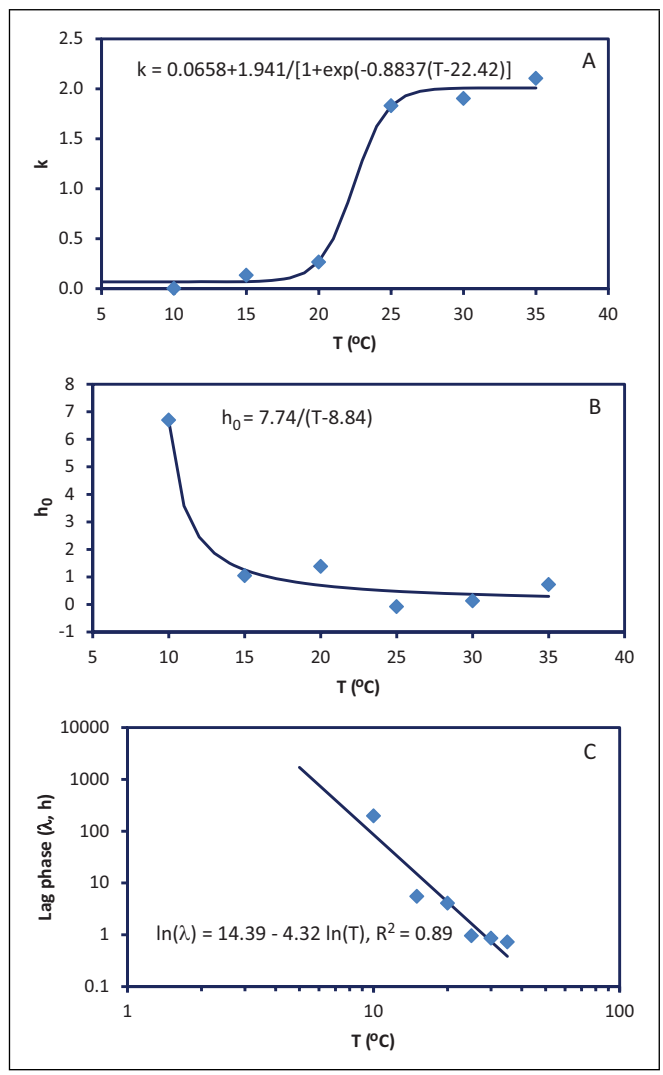

Figure 9–Effect of temperature on lag phase development –k in the Huang 2011 model, h_0 in the Baranyi model, and λ in the Huang 2008 model.

For the Baranyi model, the lag phase is determined by the relationship between h_0 and μ. The h_0 is actually a virtual parameter, representing the physiological state of bacteria. In most applications, the h_0 is assumed to be constant. For STEC bacterial cultures, the initial physiological state for STEC should be almost the same, as they were all freshly prepared cultures. The h_0 values should be relatively stable and close to each other. However, the values of h_0 are significantly affected by temperature (Figure 9B). At 10 °C, the estimated h_0 value is 6.70 (ASE = 2.0). This value is almost 10 times greater than the average h_0 (0.64) obtained at $T >$ 10 °C. A negative h_0 value was obtained at $T = 25$ °C, producing a negative calculated lag phase. As a general trend, the h_0 decreases exponentially with temperature and can be described by a simple exponential equation (Eq. 15). This study demonstrates that the physiological state (h_0) varies with temperature and increases exponentially as temperature decreases.

$$h_0 = \frac{7.74}{T - 8.84} \tag{15}$$

Out of the 3 models, the lag phase is explicitly defined in the Huang 2008 model, and is also a function of temperature (Figure 9C). The logarithm of lag phase is a linear function of the logarithm of temperature (Eq. 16, $R^2 = 0.89$).

$$\ln(\lambda) = 14.39 - 4.32\ln(T) \tag{16}$$

Conclusions

This study investigated the growth of STEC directly in raw ground beef, and examined 3 kinetic models to describe the bacterial growth under 6 different temperature conditions, ranging from 10 to 35 °C at 5 °C increments. Analytical results suggest that all 3 models are equally suitable for evaluating the growth of bacteria under constant temperature conditions. For STEC, the maximum cell density is a function of temperature. It increases exponentially with temperature, but plateaus at approximately 8.53 $\log_{10}$ cfu/g, based on the experimental observations in this study. The growth rates estimated by the 3 models are basically identical, and can be described by either a Ratkowsky square-root model or a Bělehrádek-type model (Huang 2010). The T_0 estimated by the Ratkowsky model is 6 °C, whereas the minimum temperature (T_{min}) estimated by the Bělehrádek-type model is 8.5 °C. Because the lag phase development and its duration was also affected by the incubation temperature, the effect of temperature on the lag phase development was also evaluated. Three additional mathematical equations were developed to describe the temperature dependence of k, h_0, and λ in the Huang 2011, Baranyi, and Huang 2008 models, respectively. Below the optimal growth temperature, the k value for the Huang 2011 model increases with temperature, whereas the h_0 and λ from the Baranyi and Huang 2008 models decreases with temperature. Combined with these secondary models, the mathematical growth models evaluated in this study can be used to describe the growth of STEC in ground beef at different temperature conditions.

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